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Identifying the role of soil microbes in plant invasions

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Manuscripts

1 SF Editorial

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3 **Identifying the role of soil microbes in plant invasions**

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15 Key-words: alien plants, bacteria, enemy release, invasive plant, mycorrhizal fungi,
16 pathogens, rhizosphere, soil biota.

17

18 Running title: Soil microbes and plant invasions

19 **Summary**

20 1. Understanding how invasions by exotic plant species occur has been, and still is a
21 fundamental goal in the field of invasion ecology. Recently, research focus has shifted to
22 belowground mechanisms of invasion, which has provided valuable insights into the role of
23 soil microbes in the invasion process.

24 2. Research on how soil microbes drive plant invasions has produced a multitude of potential
25 mechanisms and hypotheses, and we have taken this timely opportunity to organize these
26 hypotheses, explore interrelations among them and with other drivers of invasion, and to
27 identify areas requiring more in-depth study.

28 *Synthesis.* This special feature brings together current research on soil microbes as drivers and
29 responders of invasion, highlighting the methods that can be used to advance our
30 understanding of how, when and which soil microbes play a role in the invasion process.

31 Further advances in the field can be made by increasing realism in experiments, making wider
32 use of molecular methods and culturing of microbial isolates, and considering the importance
33 of microbes relative to other invasion drivers.

34 **Introduction**

35 Plant invasions are a major component of global environmental change. Over 13,000 plant
 36 species have been introduced and established self-sustaining populations somewhere outside
 37 of their native range (van Kleunen *et al.* 2015), and that number will likely increase with
 38 globalized trade and consequent introductions (Seebens *et al.* 2015). However, only a fraction
 39 of introduced plant species become invasive (i.e. widespread in the introduced range, *sensu*
 40 Blackburn *et al.* (2011)), and abundant at a local scale in invaded plant communities.
 41 Understanding what determines the success of these few invasive species has been, and still is
 42 a fundamental goal in the field of invasion ecology.

43 Many of the proposed mechanisms explaining plant invasions have been developed
 44 and tested with an aboveground focus, for example the enemy release hypothesis (Keane &
 45 Crawley 2002) and the evolution of increased competitive ability hypothesis (Blossey &
 46 Notzold 1995). However, the increased attention paid to the role of plant-soil interactions in
 47 plant communities and ecosystems (Wardle *et al.* 2004) has been reflected in the study of how
 48 soil microbes might act as drivers of plant invasions, and the theory behind this work has been
 49 based on achievements aboveground. The search for mechanisms explaining plant invasions
 50 belowground arguably came to the fore with the classic work by Callaway and colleagues on
 51 *Centaurea maculosa*, a perennial forb native to Eurasia and invasive in North American
 52 grasslands. Marler *et al.* (1999) showed that *C. maculosa* benefitted indirectly from
 53 mycorrhizal fungi through enhanced competitive ability against native grasses. Callaway *et al.*
 54 (2004) also demonstrated that this invasive species may benefit from leaving behind soil
 55 pathogens present in the native range.

56 Subsequently, research on the role of soil microbes (bacteria, archaea, fungi and
 57 oomycetes) in driving invasions has increased considerably. Most studies testing the role of
 58 soil microbes in plant invasions use some variant of the pot-based, plant-soil feedback (PSF)
 59 experiment (Box 1). Many PSF studies provide evidence that invasive plants interact
 60 differently with microbes in a way that promotes their growth over native plants. For
 61 example, the seminal study by Klironomos (2002) found that invasive plants performed
 62 relatively better on home than away soils compared to less abundant exotic or native species
 63 in an old field community. By comparing growth of seedlings of the invasive black cherry
 64 (*Prunus serotina*) on soils from the native (North America) and exotic (Europe) ranges,
 65 Reinhart *et al.* (2003) revealed that growth of the species is inhibited by the soil microbial
 66 community in the native range, but is promoted by the soil community in the exotic range.

Van der Putten *et al.* (2007a) showed that an exotic savannah grass (*Cenchrus biflorus*) may be invasive due to the lack of a soil-borne pathogen, but it also associated with fewer arbuscular mycorrhizal fungi. In a meta-analysis, Kulmatiski *et al.* (2008) showed that across studies, invasive exotic plants suffer less negative effects from soil biota than native species, which suggests that differences in plant-soil interactions are related to invasion success.

In a relatively short space of time, we have made great strides in exploring the potential role of soil microbes in driving plants invasions, and this research effort has generated a plethora of hypotheses and mechanisms. Now is a timely opportunity to organize those hypotheses and mechanisms, to explore how they might be interrelated with each other and other drivers of invasions, and to identify ways in which we can advance the field further. This special feature brings together current research in plant-soil microbe (bacteria and fungi) interactions and invasions and highlights approaches that can advance our mechanistic understanding of the role soil microbes play in the invasion process. Here in this preceding editorial, we aim to i) provide a non-exhaustive but comprehensive overview of the state-of the art and identify knowledge gaps, ii) organize the mechanisms by which soil microbes might drive invasions, and iii) provide suggestions for advancing the field.

Box 1. The plant-soil feedback method

Potted plant-soil feedback experiments have become a standard method of investigating plant-soil interactions and their role in plant invasions. Invasive plants are grown in often field-collected soil for a ‘conditioning’ phase. In a second experimental phase, invasive plants can be grown in soil that was either previously occupied by themselves (‘home’ soils) or by other species (‘away’ soils). Alternatively, invasive plants can be grown in ‘home’ soil that is sterilized, or unsterilized. After a set growth period, plant biomass is harvested, and the differences in plant biomass between home and away soils or unsterilized and sterilized soils are used as a measure of the net effects of soil biota on invasive plant performance. Negative effects of growing in unsterilized vs sterilized, or home vs away soils, would indicate effects of soil pathogens on plant performance outweigh effects of any mutualists; positive effects would indicate the reverse (Van der Putten, Van Dijk & Peters 1993; Bever 1994). Effects of soil biota on invasive plants are usually compared to effects on native species (Klironomos 2002; Van der Putten *et al.* 2007a; Engelkes *et al.* 2008; Chiuffo, MacDougall & Hierro 2015), non-invasive exotic species (Müller, van Kleunen & Dawson 2016), or the invasive

plant in its native range (Reinhart *et al.* 2003; Reinhart & Callaway 2004; Callaway *et al.* 2011).

State of the art

In order to organize the current state of the art, we have summarized the main mechanisms by which different soil microbes can lead to invasion in Figure 1. In theory, an exotic plant should be able to invade a community if the exotic has a greater fitness (or competitive ability) than the native species in the community (MacDougall, Gilbert & Levine 2009), and so soil microbes should only play a decisive role in the invasion if they lead to a higher exotic than native fitness. Following the distinction of Bardgett & Wardle (2003), we find it useful to separate direct and indirect routes. Direct routes involve physical interaction between a microbe and the exotic plant's roots (i.e. mutualists and pathogens; Fig. 1), and the microbe acts directly - positively or negatively- on the fitness of the exotic plant. Indirect routes include interactions between the alien species and the plant community which are mediated indirectly by soil microbes such as bacterial and fungal decomposers, and interference of mutualistic associations involving native plants (Fig. 1).

Direct routes

One of the leading explanations for the invasiveness of an exotic species is the enemy release hypothesis, ERH (arrow [1] in Fig. 1) (Keane & Crawley 2002; Liu & Stiling 2006). Specialist soil pathogens (e.g. fungi and oomycetes) that keep plants in check in their native range may be absent in the introduced range, which may then give them a competitive advantage over resident native species that still have their own specialist enemies. A classic example of how a species can be released from below ground pathogens is given by Reinhart *et al.* (2010) in which they show how *Prunus serotina* suffers from virulent oomycete pathogens (*Pythium* spp.) in the native range, while taxa present in the exotic range are less virulent.

An extension of the ERH is the evolution of increased competitive ability hypothesis (EICA; Blossey & Notzold 1995). Due to release from above or belowground enemies, selection should favour genotypes of exotic species that invest less in unused defence and more into growth, therefore increasing competitiveness against natives (arrow [2] in Fig. 1).

Some studies have found evidence for post-introduction evolution of increased competitive ability **in response to soil biota in the exotic range** (Maron *et al.* 2004; Ridenour *et al.* 2008; Barney, Whitlow & DiTommaso 2009). Te Beest *et al.* (2009) also found that biomass allocation in *Chromolaena odorata* switched towards stems in exotic range soils compared to the native range, inferring greater competitive ability. Zheng *et al.* (2015) found evidence that genotypes of the same species from the exotic range were affected less negatively by soil fungi than native range genotypes, but that biomass of exotic genotype was lower than for native ones overall (contrary to EICA). **Whether pathogen release directly increases exotic fitness, or does so via evolution of increased competitive ability, fully assessing its role in invasions does require biogeographic comparisons between the exotic and native ranges (Hierro, Maron & Callaway 2005). Future studies also need to identify the putative pathogens present in the native and exotic range, and how virulent they are, as in Reinhart *et al.* (2010).**

The accumulation of pathogens hypothesis involves generalist pathogens that infect not only the invader but also competing native plants (Eppinga *et al.* 2006). The accumulation of local generalist pathogens **should** result in increased or maintained dominance of the invader in the community if the local plant community is more susceptible to the pathogens that accumulate under the exotic invader (arrow [3] in Fig. 1). This is, in effect, a form of apparent competition. For example, tropical soils that are invaded by *Chromolaena odorata* accumulate high abundances of *Fusarium* spp., with strong negative effects on the native plant community (Mangla & Callaway 2008). **However, there is evidence that novel chemical compounds produced by this species can have allelopathic effects on native plants in invaded communities (Inderjit *et al.* 2008; see ‘Indirect routes’ below). It is unclear in general how important novel allelopathic chemicals are relative to accumulating pathogens or pathogen release for exotic invasion success. It is plausible that pathogen release and allelopathy facilitate initial invasion, while an increase in abundance and maintaining of dominance results from continued allelopathy or accumulated pathogens affecting natives, or both.**

Compared to the role of belowground enemies, mutualists (e.g. mycorrhizal fungi and N-fixing rhizobacteria) have **arguably** received less attention. Invasive species may often find new mutualist partners that have either co-invaded, or have been recruited from the native mutualist community, and that allow exotic plant establishment in other regions of the world (Richardson *et al.* 2000). **Mutualisms may be completely novel to the new environment, giving the exotic a fitness advantage over native species (arrow [4] in Fig. 1). A classic example is the invasion of Hawaiian volcanic soils by the shrub *Myrica faya*; this species has**

N-fixing actinomycete bacteria (*Frankia*) as mutualists, while N-fixing native plants are absent (Vitousek *et al.* 1987). One way that mutualists can disproportionally affect the fitness of an invasive plant is when the presence of local mutualists has more positive impacts on the invader in the exotic range than the native range (arrow [5] in Fig. 1; enhanced mutualisms hypothesis: Reinhart & Callaway 2006; Sun & He 2010). This may occur when exotic species exploit mycorrhizal associations, thus reaping the benefits of the symbiosis while escaping the mutual cost of maintaining the network (Sun & He 2010).

As with pathogen release, mutualist enhancement requires a biogeographic comparison between exotic and native ranges to be tested fully. While some work has been done to test the relative importance of pathogen *versus* mutualist-related mechanisms of invasion, further work is required to understand not only which is more important in general, but also whether importance is contingent upon other factors, such as the environment invaded. For example, we might expect mutualisms that increase nutrient and water uptake to be more important for invasion in environments with low nutrient or water availability, while pathogen release could play a greater role in more mesic environments with higher nutrient availability.

Indirect routes

Disturbance (i.e. removal of existing plant biomass) has historically been regarded as an important prerequisite for invasion (Ewel 1986; Rejmánek, Robinson & Rejmánková 1989; Hobbs & Huenneke 1992; Williamson 1996), and has been coined ‘one of the most commonly accepted truisms in the field of invasion ecology’ (Lockwood, Hoopes & Marchetti 2013; but see Moles *et al.* 2013). Disturbance events such as fire (Zedler & Scheid 1988) and mammal herbivory (Cross 1981) can promote invasions directly through vegetation removal (arrow [6] in Fig. 1), but effects may also be subtle and indirect, with a belowground component. The fluctuating resource hypothesis states that habitats with a high variability of resource availability (often as a result of frequent disturbance) will generally be more easily invaded than habitats with less variable resource levels, by invasive plants with high growth rates and a capacity to capitalize on free resources when available (Davis, Grime & Thompson 2000). Studies have shown that invasive plants take advantage of increases in and fluctuating levels of nutrient availability (Dawson *et al.* 2012; Parepa, Fischer & Bossdorf 2013). Soil nutrient availability can itself be affected by disturbance through stimulation of

the soil microbial community, leading to increases in nitrification and therefore nutrient availability, or shifts from fungal-dominated communities with slow nutrient cycling to bacterial-dominated communities with fast nutrient cycling (de Vries *et al.* 2012). Thus, disturbance could facilitate invasion indirectly, through microbe-mediated changes in nutrient availability that favour nutrient-demanding exotics (arrow [i] in Figure 1). One further indirect route that remains to be tested, could involve the break-up of mutualistic mycorrhizal networks during disturbance, upon which native plants depend. This may lead to invasion by less mycorrhizal-dependent exotics.

Novel weapons- chemical compounds that do not exist in the native plant community and that can have allelopathic effects- constitute another indirect pathway that can promote invasion (the novel weapons hypothesis, Callaway & Ridenour 2004; Zheng *et al.* 2015). Besides directly interfering with the invaded plant community (arrow [7] in Fig. 1), exotic invasive plants may also produce compounds that interfere with interactions between native species and their mutualists (arrow [8] in Fig. 1). For example it is known that some invasive species, such as garlic mustard (*Alliaria petiolata*) disrupt mutualistic association between mycorrhizal fungi and native seedlings, reducing nutrient availability for natives (Stinson *et al.* 2006; Hale & Kalisz 2012; Brouwer, Hale & Kalisz 2015). However, such effects may not persist in the longer term. Garlic mustard-invaded soils with a long invasion history (i.e. oldest invaded sites) showed recovery in richness of soil microbial communities (Lankau *et al.* 2009; Lankau 2011). This could be attributed to the observed decline in the production of allelochemicals with longer invasion history, which may reduce the impact of the invader on soil microbial communities (Lankau *et al.* 2009; Lankau 2010).

It is not known if and how allelochemicals have strong effects on other soil fungi such as decomposers, with consequences for nutrient cycling and subsequent invasion success. For example, if allelopathy reduces fungal decomposer abundance, this could result in more bacterial-dominated decomposer communities, leading to higher nutrient availability that could promote invasion (arrow [ii] in Figure 1). A different connection between novel weapons and another invasion driver (enemy release) has, however, been revealed in *Chromolaena odorata* by Zheng *et al.* (2015). They found that exotic genotypes have lower defence against aboveground herbivores (from which the species may be released), but greater defence against belowground pathogens than native genotypes. The species also appears to have evolved greater concentrations of allelopathic compounds in plant tissues which reduce

the growth of native plants in the exotic range (Zheng *et al.* 2015). It remains to be seen if evolution of enhanced novel weapons could also result in general from soil pathogen release.

Advancing the field

We have highlighted a number of ways in which soil microbes can result in fitness advantages for exotic plants and subsequently in invasion. A number of these routes require further study, in particular the indirect routes (Fig. 1). Among the direct routes, the role of soil-borne pathogens in invasions has received much research attention, and we suggest that more studies in the future could explicitly tested the importance of mutualist enhancement *versus* pathogen release. In addition, we believe that in order to advance our understanding of how soil biota drive invasions further, we need to consider how our conceptual and methodological approaches can be improved. Below we discuss promising approaches that have been put into practice by the articles in this special feature and we provide additional directions for future research. These fall into two main categories: 1) achieving greater realism in our experiments, and 2) identifying putative players in plant-soil interactions.

1) Greater realism in experiments

i) Moving into the field (or moving the field into the greenhouse)

Much of our understanding of plant-soil microbe interactions, in general as well as in the context of invasions, has been obtained from glasshouse-based, plant-soil feedback experiments in pots. While this approach is a valuable one, we need more experiments conducted under field conditions in order to better understand the role of soil microbes in invasions. Schittko *et al.* (2016) demonstrate the importance of this by showing that plant-soil feedback effects observed for plants in a controlled greenhouse experiment are not replicated for plants placed in the field. Instead, this study shows that plant-soil feedback effects that were largely positive in a greenhouse, were largely non-existent in a field setting. Thus, it is possible that an inference made about invasions from PSF effects measured under greenhouse conditions may not hold in the field.

Manipulating soil microbes without affecting other soil properties is difficult to achieve, whether in the field using biocides or in pot experiments using sterilisation and

inoculation. Pot-based PSF experiments that typically involve mixing soils and adding a small amount of sterilized or unsterilized inoculum to a background sterile soil reduce realism by changing the soil physical structure and potentially altering the microbial community. One way to make pot-based experiments more realistic would be to use soil cores or turfs with intact structure. Comparing plants grown on home soils to those grown on away soils will include abiotic as well as biotic differences that occur in the field. If differences in soil microbial communities and in physicochemical properties can be established between home and away soils, then structural equation modelling could be used as in Collins *et al.* (2016), to assess how important abiotic and biotic components are for plant-soil feedbacks. Using intact soils is not a new idea (Diez *et al.* 2010), but we think combining this with structural equation modelling could yield more realistic inferences about the importance of soil microbes in plant-soil feedback experiments. However, this approach would still treat the soil as a ‘black box’, as the specific agents of plant-soil feedback effects and their function would not be identified.

ii) Measuring fitness-related parameters

Besides increasing realism, moving the study of plant-soil interactions and invasions into the field could also supplement our methodological toolbox, through measurements of fitness-related measurements of plant performance. The studies by Müller *et al.* (2016) and Schrama & Bardgett (2016) assess establishment of plants introduced to grassland communities in Germany and the UK respectively. Müller *et al.* assess the effects of changes in soil microbial communities through biocide treatment, and Schrama and Bardgett utilise differences in microbial community structure associated with different land use and vegetation type. Both studies consider the importance of the belowground component of biotic resistance, a concept which hitherto has been approached largely from an aboveground (plant community composition) perspective. Future studies could assess how plant-soil microbe interactions affect other aspects of plant performance, such as reproduction and plant survival over time.

iii) Using spatially and temporally explicit experimental designs

Invasions are temporally dynamic, spatially explicit events, but our attempts to understand how soil microbes can drive invasions rarely take the spatial and temporal components into account. One exception is a study by Dostàl et al. (2013), who showed that plants of giant hogweed (*Heracleum mantegazzianum*) grown on soils occupied by older hogweed populations experienced more negative PSF effects than plants grown on soils from younger hogweed populations. Pot-based studies could improve the ecological realism of experiments by spatially linking them to invasions occurring in the field, through sampling of soils from different invader populations. Alternatively, to capture the role of soil microbes in invasion as a dynamic process, soils could be sampled across an invasion front within a population and in neighbouring uninvaded vegetation. Combining measurements of plant-soil feedback effects of natives and invasives using soils at different locations, with measurements of plant performance and fitness across the population in the field, would allow us to predict how the invasion will proceed over time and across space. For example, both the invader and natives could be grown in these soils, and if the invader is less negatively affected by soil microbes in all soils than the native species, then we would expect the invasion to expand and exclude the natives. If the invasion fails to expand, this would indicate that the effects of soil microbes on the invasion are contingent upon other factors.

iv) Considering the importance of soil microbes in the context of other factors

Schittko et al. make the point that the importance of soil microbes to invasions needs to be considered in **the context of other factors**, similar to the importance of plant competition in shaping communities (Brooker *et al.* 2005). **In addition, the end-result of plant-soil interactions may be contingent upon effects that extend beyond plants to their herbivores, and to herbivore predators. For example, Bennett *et al.* (2016) show that aphids had increased performance on *Solanum* plants inoculated with AMF, as did their parasitoid wasp predators. Performance of *Solanum* varied, however, according to plant and aphid genotype. Thus, effects of plant-microbial interactions on invasions may vary among populations and genotypes of invasive plants. Quantifying this variation and identifying the cause of it will help us to understand when soil microbes are most likely driving an invasion.**

Disturbance is considered key for invasions to occur (Rejmánek, Robinson & Rejmánková 1989; Hobbs & Huenneke 1992) but its role relative to soil microbes and aboveground enemies (herbivores) has only been tested for the first time in the study by

Müller et al., in a field setting. Regardless of the system, species, and type of soil microbes that are focused on, soil microbes are unlikely to act in isolation from other factors. Schrama and Bardgett show that drought disturbance can make grasslands more vulnerable to invasion by exotic plants, but only if they are disturbed, low diversity grasslands with bacterial-dominated soil communities (and not high diversity, fungal dominated grasslands). Thus, we need to consider how important changes in soil microbial communities are in driving invasions, compared to other effects of disturbance, and this will likely depend upon the nature of the existing plant and soil microbial communities.

2) Identifying putative players in plant-soil interactions

i) Biogeographic comparisons

Comparing soil microbial communities in native range and exotic range soils can indicate whether mutualists, pathogens or both are likely involved in driving an invasion, especially when combined with a PSF experiment approach (Callaway *et al.* 2011). While it is recognised that a lack of mutualists could limit an invasion in the exotic range, Shelby *et al.*, (2016) point out that we know little about how the effects of interactions with mutualists differ between native and exotic ranges. They address this by measuring the growth of *Trifolium* plants from native (European) and exotic (New Zealand) populations growing on native and exotic range soils, and quantifying parasitic rhizobia nodule formation and arbuscular mycorrhizal fungal (AMF) colonization of roots. Shelby et al. find evidence in support of reduced mutualism in the exotic range, and variation in the effects of rhizobia and AMFs among *Trifolium* species. However, this does not translate to differences in invasion success among *Trifolium* species, which highlights that differences in plant-microbial associations occurring between ranges needs to be clearly linked to invasion success for there to be sufficient evidence for a role in invasion.

ii) Wider use of the molecular toolbox

Plant-soil feedback experiments often do not include identification of the soil microbes responsible for driving the observed PSF effects on plant growth. Next generation sequencing methods (Illumina, 454-pyrosequencing) are becoming cheaper and more accessible, and this should help to identify the microbes present in soils and roots of invasive

plants in the native and exotic ranges that may be responsible for plant-soil feedback effects, and ultimately that may drive or limit an invasion. For example, Gundale *et al.* (2016) use next-generation sequencing methods (454-pyrosequencing) combined with a biogeographic approach to show that fungal pathogen release is not a likely explanation for north American *Pinus contorta* invasion success in Europe and New Zealand. In addition, Gundale *et al.* show that invasion for this tree species beyond initial establishment is not limited by a lack of mutualist ectomycorrhizal fungi when co-invasion by introduced fungi occurs, or when the native vegetation is phylogenetically closely related. By comparing soil microbial communities both between native and exotic ranges, and among sites within exotic ranges in this way, we can begin to assess how important different components of soil microbial communities are likely to be for invasion success.

Next-generation sequencing can also be employed to better understand how soil microbes respond to an invasion. Collins *et al.* (2016) investigate the impacts of a range-expanding native plant (sagebrush) on soil microbial communities, and found that sagebrush invasion increased microbial diversity and changed composition. However, Collins *et al.* also find that bacterial composition returned to a state similar to open sites four years after sagebrush removal. Thus, next generation sequencing can be used to assess soil legacy effects of invasions after invader removal.

iii) Culturing microbial isolates

While next-generation sequencing can identify thousands of taxa that are associated with the roots and root zones of invasive plants, it cannot by itself identify the nature of the interaction between each taxon and the plant. In order to understand whether soil microbes function as pathogens, mutualists or neither for a target plant, we need to be able to isolate microbes, prepare pure cultures and inoculate plants with them in order to assess how they affect plant performance (i.e. fulfilment of Koch's postulates, as suggested by van der Putten, Klironomos & Wardle (2007b)). Klironomos (2002) and Callaway *et al.* (2011) achieved this partially, by filtering soil solutions to separate out AMFs from saprobe and pathogenic fungi. Klironomos (2003) went further by isolating individual species and genotypes of AMFs, reinoculating plants and measuring their growth response. Similar approaches have been used to isolate and identify foliar pathogens of the invasive grass *Microstegium vimineum* in the USA (Stricker *et al.* 2016), and could be adapted to culture root pathogens of invasive plants.

Given the massive diversity of bacteria and archaea, and that many fungi and oomycetes are known mutualists and pathogens, we could prioritise in-depth studies involving isolation and reinoculation of model invasive plants with fungi and oomycetes. Such in-depth studies will be challenging, as they will require the building of isolated and cultured microbe libraries. Not all microbes will be successfully cultured, and multiple combinations of inoculation will be necessary to assess effects of one microbe on a plant in the presence of others that would naturally occur. However, microbial isolation and inoculation could be used in combination with NGS approaches and existing gene databases in order to target those taxa most likely to act as pathogens or mutualists, based on existing information of ecological roles (as in Gundale *et al.* 2016).

Concluding remarks

We have made great progress in the study of plant-soil microbe interactions and invasions, and multiple mechanisms and microbial players have been identified. In order to advance the field further, we require sophisticated approaches, carefully designed experiments and in-depth studies of model systems that account for these multiple potential mechanisms and the spatiotemporal dynamics of plant invasions. By utilising molecular tools, making biogeographic comparisons, conducting PSF experiments in a more realistic manner, and by considering the role of soil microbes in the context of other factors, the studies in this special feature have begun to identify when, which and how soil microbes can drive and respond to plant invasions.

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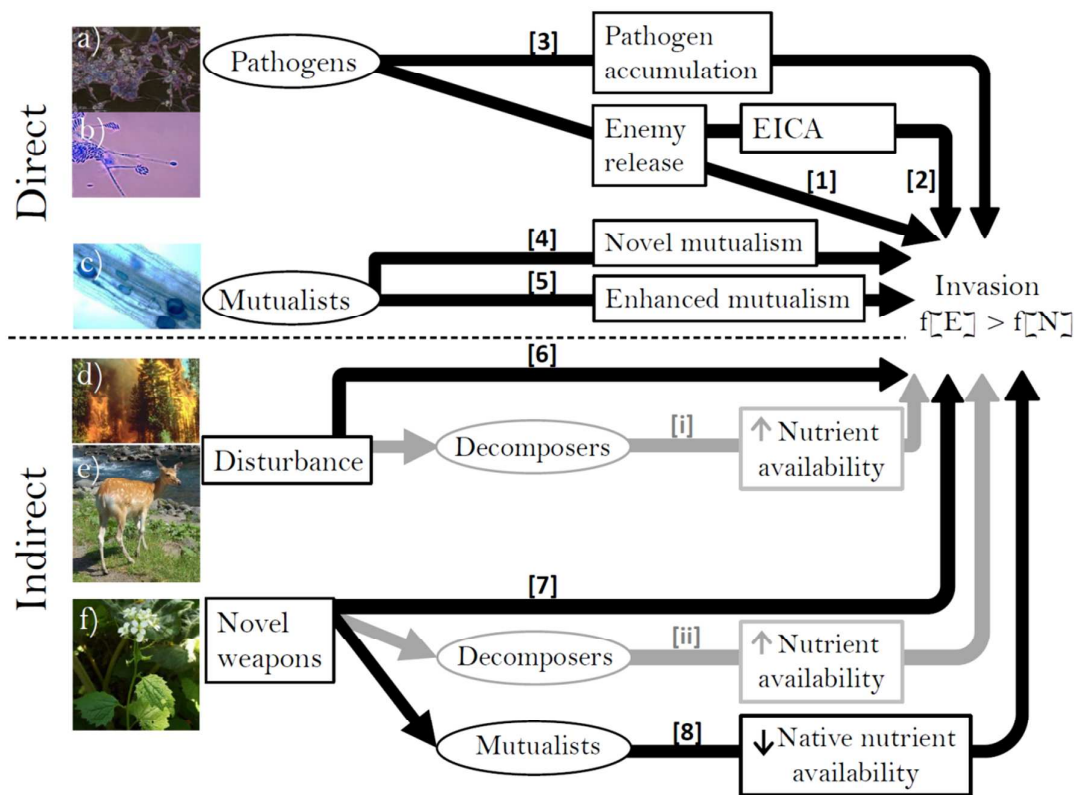
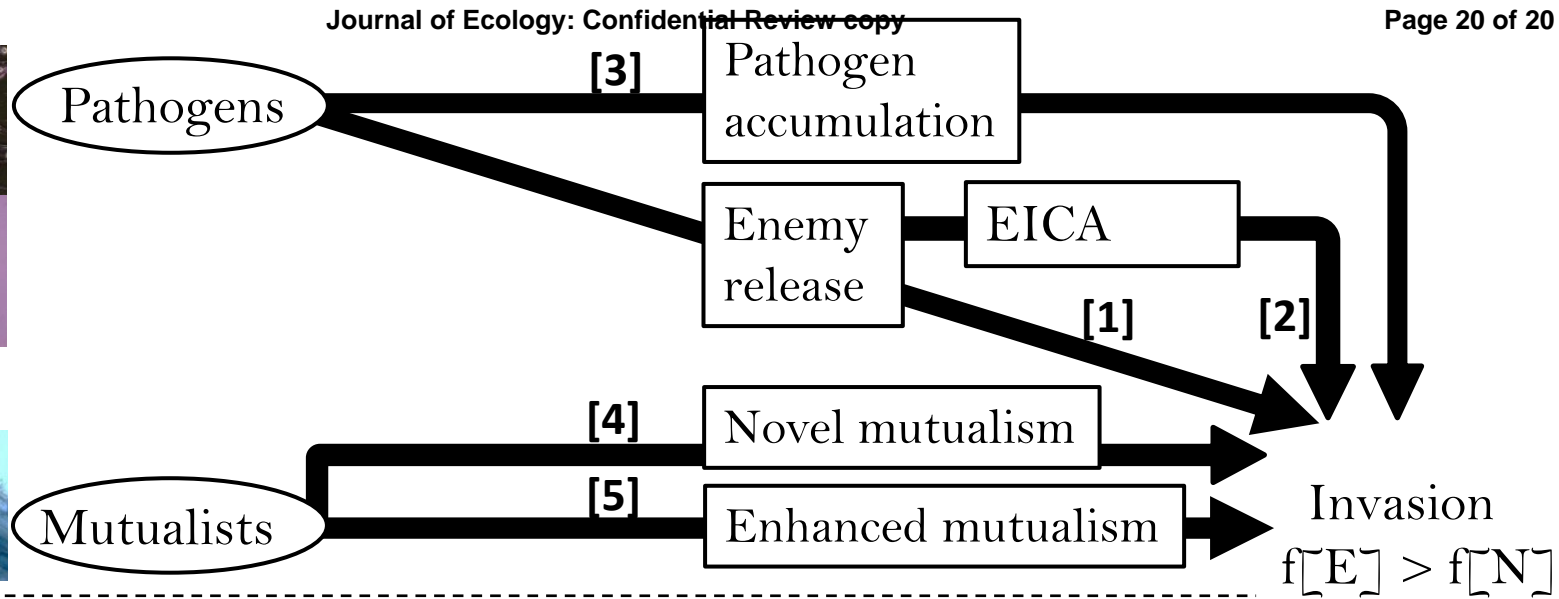
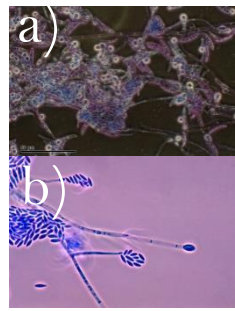


Figure 1. Direct (top) and indirect (bottom) routes to invasion via soil microbes, which should involve greater fitness of exotics ($f[E]$) than natives ($f[N]$). Pictures illustrate examples of direct and indirect routes. Pathogens such as (a) *Pythium* spp. may be less virulent or absent in the exotic range leading to enemy release [1] or evolution of increased competitive ability (EICA) [2]. Alternatively, accumulated pathogens such as *Fusarium* spp. (b) in invader-occupied soils may have a strong negative effect on native plant fitness [3]. Mutualistic microbes such as arbuscular mycorrhizal fungi (c) may provide novel [4] or enhanced [5] mutualistic advantages to exotics over natives. (d) Disturbances such as fire and (e) herbivory by large mammals could promote invasion directly [6] or indirectly [7] through microbial changes in nutrient cycling [i]. Novel weapons can directly suppress natives (7), or indirectly as in (f) Garlic mustard (*Alliaria petiolata*), through suppression of native mutualists [8]. Novel weapons could also affect decomposers and nutrient cycling, in theory [ii]. Ellipses = microbial players, boxes = hypotheses and mechanisms. Black arrows and numbers = well-studied mechanisms, grey arrows and letters = less-studied routes. Photo credits: (a) Josef Reischig, CSc. Creative Commons SA 3.0; (b) CDC/ Libero Ajello, Creative Commons CC0; (c) W. Dawson; (d) Bureau of Land Management, Creative Commons CC0; (e) Anon., Creative Commons Attribution-Share Alike 3.0 (f) M. Schrama.

Direct



Indirect

